#5

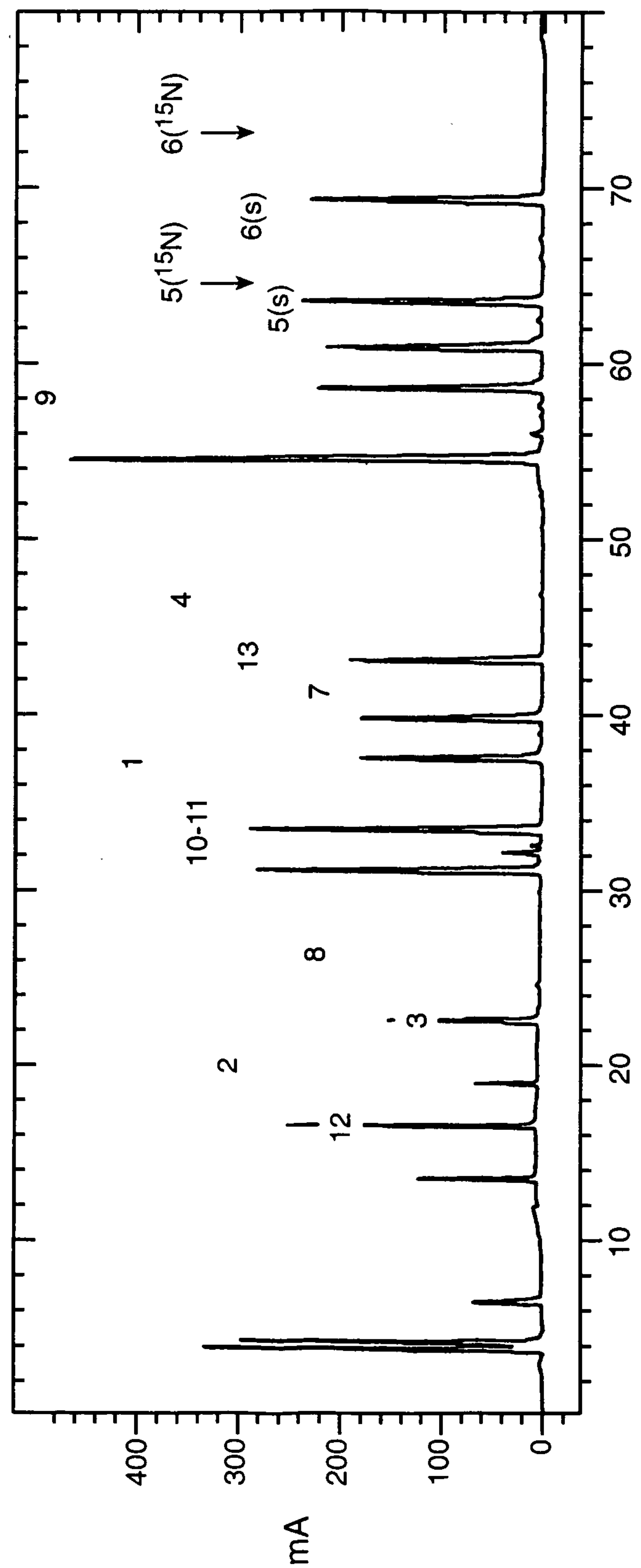

mA
0
100
200
300
400
10
20
30
40
50
60
70
12
2
3
8
10-11
1
7
13
4
9
5(s)
5(^{15}N)
6(s)
6(^{15}N)

FIG._1

#5

FIG._2A

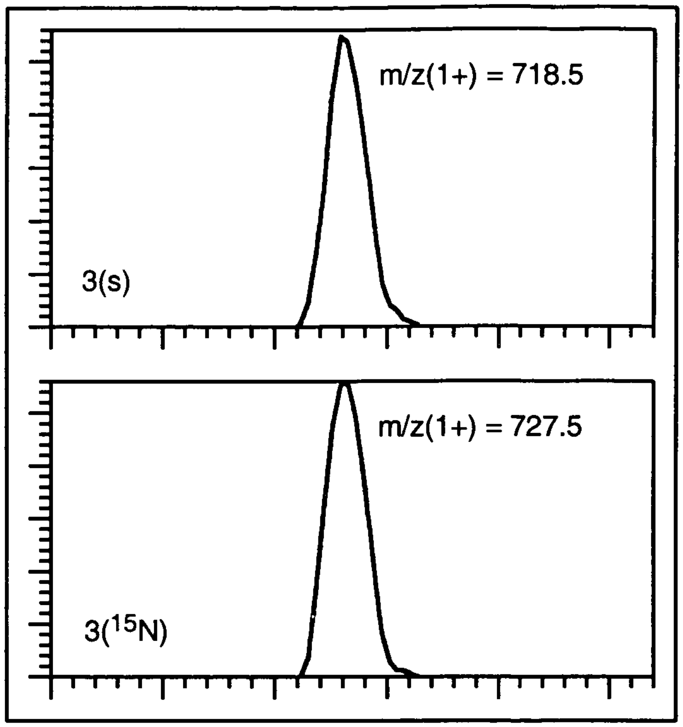
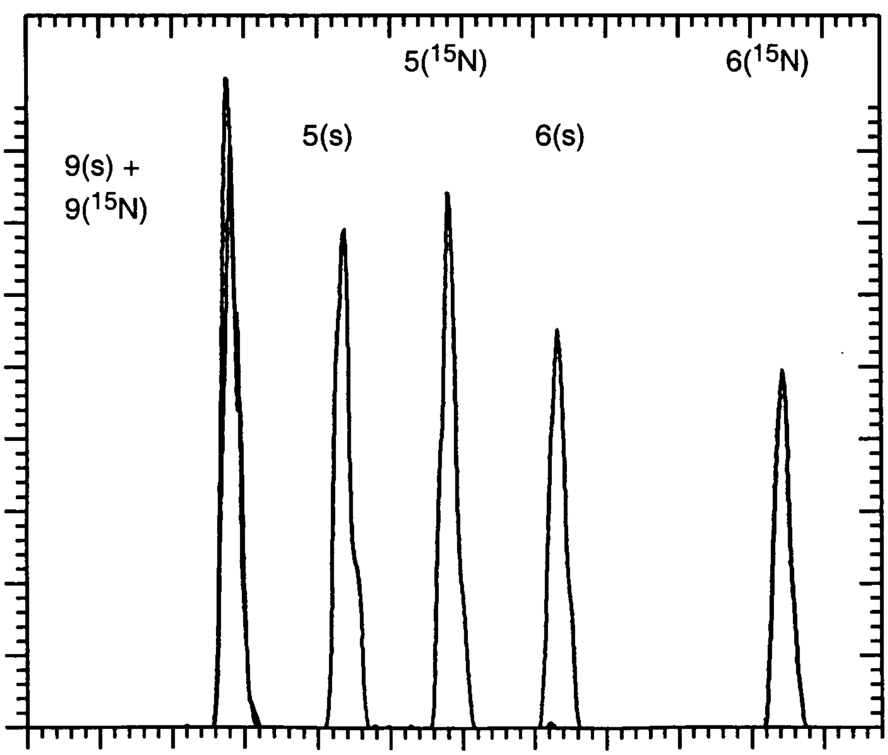


FIG._2B



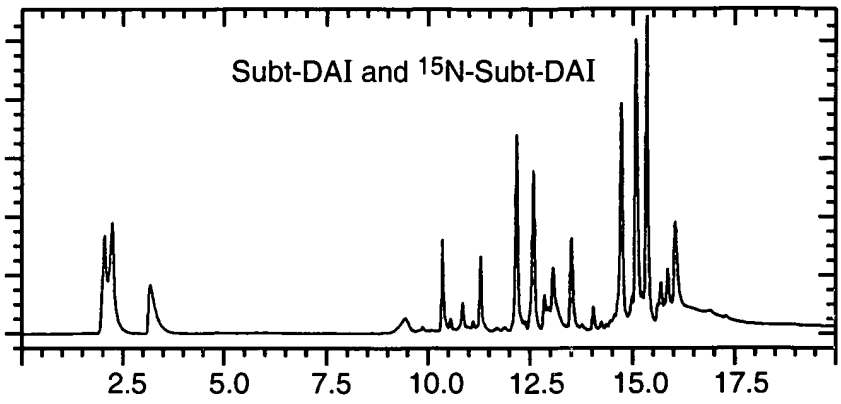


FIG._3

FIG._4A

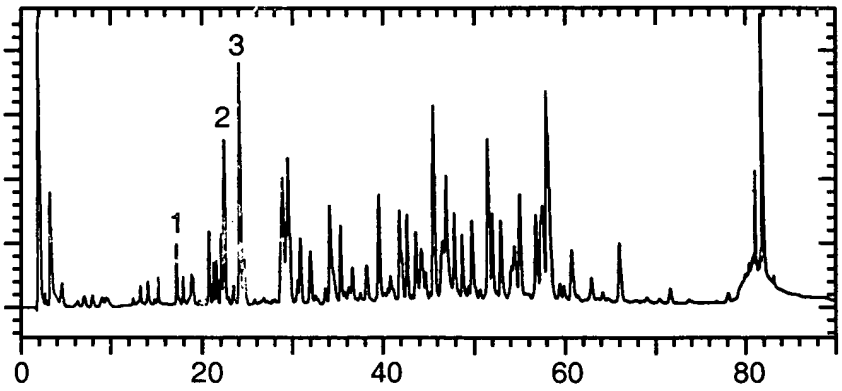


FIG._4B

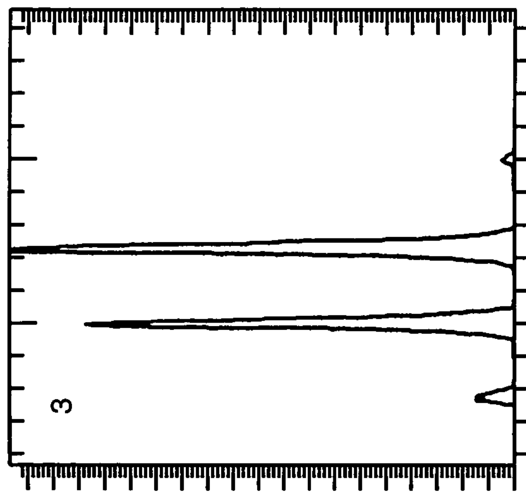


FIG._5(3)

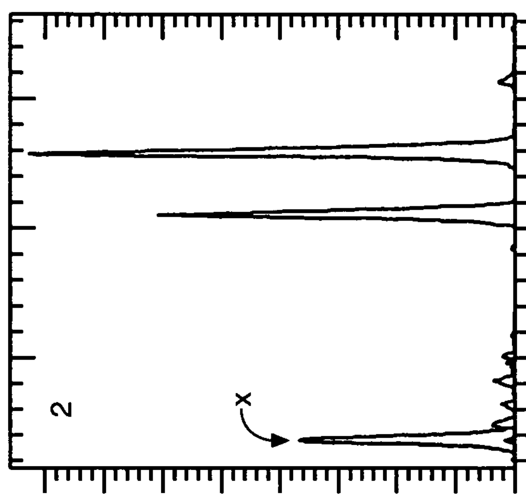


FIG._5(2)

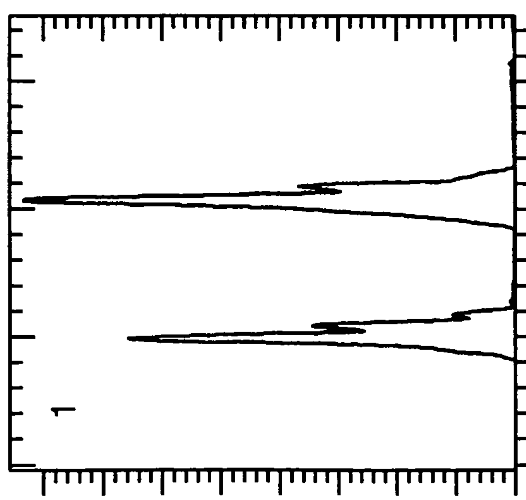


FIG._5(1)

Pep #	Sequence	m/z (wt)	m/z (^{15}N)	TIC Peak Area Ratio	UV Peak Area Ratio
1	AQSVPWGISR	1100.58(1+)	1115.53(1+)	1.013	
2	VQAPAAHNR	482.25(2+)	490.23(2+)	1.028	
3	GLTGSGVK	718.40(1+)	727.38(1+)	1.033	
4	VAVLDTGISTHPDLNIR	911.49(2+)	922.45(2+)	0.997	
5	GGASFVPGEPSTQDGNGHGTHVAGTIAALDNSIGVLGVAPSAELYAVK	1531.09(3+)	1549.71(3+)	1.049	
5 (subtilisin)	N	1530.77(3+)	-		0.981
6	VLGASGSGAISSIAQGLEWAGNNGMHVANLSLGSPSPSATLEQAVNSATSR	1642.14	1663.08(3+)	0.979	
6 (subtilisin)	SV	1642.80(3+)	-		1.003
7	GVLVVAASGNSGAGSISYPAR	967.51(2+)	979.47(2+)	1.042	
8	YANAMAVGATDQNNNR	855.38(2+)	867.34(2+)	0.971	
9	ASFSQYGAGLDIVAPGVNVQSTYPGSTYASLNGTSMATPHVAGAAALVK	1600.46(3+)	1619.07(3+)	1.010	
10-11	QK NPSWSNVQIR	729.38(2+)	739.35(2+)	1.044	
12	NHLK	511.29(1+)	519.27(1+)	1.021	
13	NTATSLGSTNLYGSGLVNAEAATR	1185.08(2+)	1200.04(2+)	1.028	
		average peak area ratio:		1.018 ±2.5%	
		average peak area ratio:			0.992 ±1.6%
		average ratio of both methods:		1.005 ±1.3%	
		intended ratio:		1.000	

FIG._6

Table II. Ratio of Concentration and Catalytic Activity (Conversion Factor) of 13 Variants Generated from Subtilisin-DAI and Expresssed in Microtiter Plates[1]

	Conversion by Peptide Mapping with ^{15}N-Internal Protein Standard		
Variant	EL3.16	OS36.7	EL3.17
Clone 1	0.035	0.015	0.021
Clone 2	0.037	0.014	0.021
Clone 3	0.035	0.015	0.019
Clone 4	0.038	0.014	-
Clone 5	0.038	0.014	-
	Conversion by MBI Titration		
Clone 1	0.036	0.015	0.020

FIG._7

[1]Clones within the three groups, EL3.16, OS36.7, and EL3.17 had the same sequence. Activity was measured by the suc-AAPF-pNA assay (Hsia et al., 1996). The concentration was measured by the peptide mapping method with ^{15}N-labeled subtilisin-DAI as internal standard. The range of concentrations was 2 to 5 $\mu g \cdot ml^{-1}$. The conversion factor was verified by an active site titration with a mung bean inhibitor (MBI) solution calibrated on the same plate with a previously calibrated solution of subtilisin-DAI (Hsia et al., 1996).